CLAIMS

WE CLAIM:

1. An aPL analog that binds specifically to B cells to which an aPL epitope binds.

2. The analog of claim 1 wherein the analog lacks a T cell epitope.

3. The analog of claim 1 wherein the analog is a peptide.

4. The analog of claim 3 wherein the peptide comprises the sequence CLILAPDRC, CLILTPDRC, CLLLAPDRC, CTILTLDRC, CLVLALDRC, CTILTPDRC, CILLAHDRC, CGNAADARC, CTNWADPRC, CGNIADPRC, CTNLTDSRC, CGNPTDVRC, GILLNEFA, GILTIDNL, GILNALDYV, LSDPGYVRNIFH or LTDPRYTRDISNFTD.

5. The analog of claim 3 wherein the peptide comprises the sequence AGPCLGVLGKLCPG, GPCLGVLGKLCPG, PCLGVLGKLCPG, CLGVLGKLCPG, AGPCLGVLGKLCG, CLGVLGKLC, GPCILLARDRCG or AGPILLARDRCPG.

6. The analog of claim 3 wherein the peptide contains at least one proline and further wherein α-methyl proline is substituted for at least one said proline.

7. The analog of claim 3 wherein a D-amino acid is substituted for at least one L-amino acid.

8. The analog of claim 3 wherein the peptide is cyclized by a disulfide bond.

9. The analog of claim 8 wherein a thioether bond is substituted for the disulfide bond.

10. The analog of claim 3 wherein the peptide contains at least one leucine and further wherein isoleucine is substituted for at least one said leucine.

11. A composition for inducing specific B cell tolerance to an aPL immunogen comprising a conjugate of a nonimmunogenic valency platform molecule and an aPL antibody-binding analog that (a) binds specifically to B cells to which an aPL immunogen binds and (b) lacks the T cell epitope(s) of the immunogen.

12. The composition of claim 11 wherein the aPL antibody-binding analog is a peptide comprising the sequence CLILAPDRC, CLILTPDRC, CLLLAPDRC, CTILTLDRC, CLVLALDRC, CTILTPDRC, CILLAHDRC, CGNAADARC, CTNWADPRC, CGNIADPRC, CTNLTDSRC, CGNPTDVRC, GILLNEFA, GILTIDNL, GILNALDYV, LSDPGYVRNIFH or LTDPRYTRDISNFTD.

13. The composition of claim 11 wherein the aPL antibody-binding analog is a peptide comprising the sequence AGPCLGVLGKLCPG, GPCLGVLGKLCPG, PCLGVLGKLCPG, CLGVLGKLCPG, AGPCLGVLGKLCG, CLGVLGKLC, GPCILLARDRCG or AGPILLARDRCPG.

14. The composition of claim 11 wherein the aPL antibody-binding analog is an analog according to claim 6.

15. The composition of claim 11 wherein the aPL antibody-binding analog is an analog according to claim 7.

16. The composition of claim 11 wherein the aPL antibody-binding analog is an analog according to claim 8.

17. The composition of claim 11 wherein the aPL antibody-binding analog is an analog according to claim 9.

18. The composition of claim 11 wherein the aPL antibody-binding analog is an analog according to claim 10.

19. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises triethylene glycol.

20. The composition of claim 19 wherein the valency platform molecule comprises AHAB-TEG.

21. The composition of claim 19 wherein the valency platform molecule comprises compound 46, A-DABA-ATEG.

22. The composition of claim 19 wherein the valency platform molecule comprises compound 51, A-PABA-DT-TEG.

23. The composition of claim 19 wherein the valency platform molecule comprises compound 55, MP-TEG.

24. The composition of claim 19 wherein the valency platform molecule comprises compound 60, A-PIZ-IDA-TEG.

25. The composition of claim 19 wherein the valency platform molecule comprises compound 68, A-PIZ-IDA-HB-TEG.

26. The composition of claim 19 wherein the valency platform molecule comprises compound 72, A-PIZ-HIP-TEG.

27. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises polyethylene glycol.

28. The composition of claim 28 wherein the valency platform molecule comprises DABA-PEG.

29. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises tetraaminobenzene.

30. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises heptaaminobetacyclodextrin.

31. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises tetraaminopentaerythritol.

32. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises 1,4,8,11-tetraazacyclotetradecane (Cyclam).

33. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises 1,4,7,10-tetraazacyclododecane (Cyclen).

34. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises compound 63, tetrakis-A-PIZ-PMA.

35. The composition of claim 11 wherein the nonimmunogenic valency platform molecule comprises compound 55, MP-TEG.

36. The composition of claim 11 wherein the conjugate is derived from tetrakis-BMB.

37. A non-immunogenic valency platform molecule comprising AHAB-TEG.

38. A non-immunogenic valency platform molecule comprising compound 46, IA-DABA-ATEG.

39. A non-immunogenic valency platform molecule comprising compound 51, BA-PABA-DT-TEG.

40. A non-immunogenic valency platform molecule comprising compound 55, BMP-TEG.

41. A non-immunogenic valency platform molecule comprising compound 60, BA-PIZ-IDA-TEG.

42. A non-immunogenic valency platform molecule comprising compound 68, BA-PIZ-IDA-HB-TEG.

43. A non-immunogenic valency platform molecule comprising compound 72, BA-PIZ-HIP-TEG.

44. A non-immunogenic valency platform molecule comprising compound 63, tetrakis-BA-PIZ-PMA.

45. A method of treating an individual suffering from an aPL antibody-mediated disease comprising administering an effective amount of the composition of claim 11 to an individual in need thereof.

46. The method of claim 45 wherein said aPL antibody-mediated disease is stroke.

47. The method of claim 45 wherein said aPL antibody-mediated disease is fetal loss.

48. The method of claim 45 wherein said aPL antibody-mediated disease is antiphospholipid antibody syndrome (APS).

49. The method of claim 45 wherein said aPL antibody-mediated disease is primary antiphospholipid antibody syndrome (PAPS).

50. The method of claim 45 wherein said aPL antibody-mediated disease is thrombosis.

51. A method for identifying analogs of epitopes which specifically bind aPL antibodies isolated from humans suffering from an aPL antibody-mediated disease comprising:

(a) preparing phage random peptide libraries;

(b) screening said libraries with aPL antibodies to identify aPL mimetic epitopes, wherein said screening comprises

(i) screening said libraries by biopanning;

(ii) further screening phage isolated by biopanning in (i) by micropanning; and

(iii) identifying phage containing aPL antibody high-affinity binding peptides recovered in (ii) by immunoassay.

52. A method of biopanning phage random peptide libraries to identify and isolate peptides which bind to aPL antibody comprising:

(a) reacting affinity-purified aPL antibody with phage bearing random peptide inserts;

(b) recovering phage bearing random peptide inserts which bind to the aPL antibody;

(c) infecting a microorganism with phage recovered in (b); and

(d) culturing the infected microorganism in an antibiotic-containing medium in order to isolate the phage.

53. A method of micropanning phage random peptide libraries to identify and isolate peptides having a high binding affinity to aPL antibodies comprising:

(b) isolating phage bearing random peptide inserts by biopanning;

(b) incubating the phage recovered in step (a) in microplate wells coated with aPL antibody bound to Protein G;

(c) washing the microplate wells to remove unbound phage;

(d) eluting bound phage; and

(e) infecting a microorganism with phage recovered in (d); and

(f) culturing the infected microorganism in an antibiotic-containing medium in order to isolate the phage.

54. The method of claim 51 wherein the immunoassay is a phage-capture ELISA comprising:

(a) incubating phage bearing random peptide inserts isolated by micropanning in the microplate wells coated with aPL antibody;

(b) washing away unbound phage;

(c) incubating a labeled anti-phage antibody to the wells;

(d) washing away unbound labeled anti-phage antibody;

(e) adding a label substrate; and

(f) measuring signal development of the substrate to identify high affinity-binding phage.

55. The method of claim 54 wherein the label is an enzyme.

56. Then method of claim 54 wherein the substrate is colorimetric.

57. The method of claim 54 further comprising performing an additional phage-capture ELISA assay of the high affinity-binding phage comprising:

(a) coating a uniform amount of the phage on microplate wells;

(b) incubating aPL antibody in the wells,

(c) washing away unbound antibody,

(e) incubating a labeled anti-aPL antibody with the bound aPL antibody;

(f) washing away unbound labeled anti-aPL antibody;

(g) adding a substrate to the wells; and

(h) measuring signal development of the substrate to measure the relative binding affinity of the phage.

58. The method of claim 57 wherein the label is an enzyme.

59. The method of claim 57 wherein the substrate is colorimetric.

60. The method of claim 51 wherein the immunoassay is a colony-blot immunoassay comprising:

(a) culturing a microorganism infected with phage bearing random peptide inserts on a membrane atop an agar-containing culture medium;

(b) replicate transferring the microorganism cultured in (a) by blotting the microorganism on a membrane atop an agar-containing culture medium;

(c) incubating the transferred microorganism;

(d) lysing the microorganism;

(e) digesting the microorganism;

(f) blocking the membrane;

(g) incubating the membrane with aPL antibody;

(h) washing away unbound aPL antibody;

(i) incubating a labeled anti-aPL antibody with the membrane;

(j) washing away unbound labeled anti-aPL antibody;

(k) adding a substrate; and

(l) measuring signal development of the substrate to identify high affinity-binding phage.

61. The method of claim 60 wherein the membrane is nitrocellulose.

62. The method of claim 60 wherein the microorganism is digested with lysozyme.

63. The method of claim 60 wherein the blocking solution is gelatin.

64. The method of claim 60 wherein the label is an enzyme.

65. The method of claim 60 wherein the substrate is colorimetric.

66. A method for assaying and ranking for affinity-binding characteristics epitopes which specifically bind aPL antibodies isolated from humans suffering from an aPL antibody-mediated disease is also encompassed, the method comprising:

(a) coating wells of a microtitration plate with cardiolipin;

(b) adding adult bovine or human serum as a source of β2-GPI to bind to the cardiolipin and to prevent non-specific binding to the wells of the plate;

(c) incubating a solution of monomeric analog and a high-titered aPL antibody for a pre-determined time;

(d) adding the aPL antibody/analog mixture to wells of the microtitration plate and incubating for a pre-determined time;

(e) washing the wells to wash away unbound aPL antibody;

(f) adding anti-human IgG conjugated with a label to the wells of the plate and incubating for a pre-determined time;

(g) washing the wells to wash away unbound anti-human IgG conjugate;

(h) adding a substrate for the labeled conjugate and developing the substrate/label reaction for a pre-determined time;

(i) measuring the end-product of the substrate/label reaction to quantitate the amount of aPL antibody bound to the well;

(j) calculating the percentage inhibition, if any, of binding of the aPL antibody to determine the affinity of the analog to the aPL antibody.

67. The method of claim 66 wherein the conjugate is labeled with an enzyme.

68. The method of claim 66 wherein the substrate is colorimetric.

69. A diagnostic immunoassay for determining the presence of aPL antibody in body fluids taken from subjects suspected of suffering from an aPL antibody-mediated disease comprising

(a) contacting a sample of a body fluid with an analog of an epitope which specifically binds aPL antibodies

(b) detecting aPL antibodies bound by the analog.

70. The immunoassay of claim 69 wherein the immunoassay comprises:

(a) coating wells of a microtitration plate with an analog of an epitope which specifically binds aPL antibodies;

(b) washing the wells to wash away unbound analog;

(c) adding a test sample of a body fluid to the wells and incubating for a pre-determined time;

(d) washing the wells to remove unbound test sample;

(e) adding anti-human IgG conjugated with a label to the wells of the plate and incubating for a pre-determined time;

(f) washing the wells to wash away unbound anti-human IgG conjugate;

(g) adding a substrate for the labeled conjugate and developing the substrate/label reaction for a pre-determined time;

(h) measuring the end-product of the substrate/label reaction to determine the presence of anti-aPL antibody in the test sample.

71. The immunoassay of claim 70 wherein the label is an enzyme and the substrate is colorimetric.

72. Hydrophilic linkers for connecting peptides or other bioactive molecules to valency platform molecules with the formula

$$R^1S(CH_2CH_2O)_nCH_2CH_2O(CH_2)_mCO_2R^2$$

wherein n = 0-200, m = 0 to 10, R^1 = H or a protecting group such as trityl, R^2 = H or alkyl or aryl, such as 4-nitrophenyl ester.

73. The linkers of claim 72 wherein m = 0 to 2.

74. The conjugate of claim 11 wherein the aPL analog is bound to the nonimmunogenic valency platform molecule by a sulfhydryl containing moiety.